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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68, C07H 21/04, C12N 9/24, 15/56	A1	(11) International Publication Number: WO 97/12991
		(43) International Publication Date: 10 April 1997 (10.04.97)

(21) International Application Number: PCT/CA96/00627

(22) International Filing Date: 20 September 1996 (20.09.96)

(30) Priority Data:
60/004,157 22 September 1995 (22.09.95) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published*With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: METHOD FOR ISOLATING XYLANASE GENE SEQUENCES FROM SOIL DNA, COMPOSITIONS USEFUL IN SUCH METHOD AND COMPOSITIONS OBTAINED THEREBY

(57) Abstract

Xylanase DNA is recovered from soil by PCR amplification using degenerate primers. Because of the complexity of the soil samples, it is likely that the recovered product will include more than one species of polynucleotide. These recovered copies may be cloned into a host organism to produce additional copies of each individual species prior to characterization by sequencing. Recovered DNA which is found to vary from known xylanases can be used in several ways to facilitate production of novel xylanases for industrial application. First, the recovered DNA, or probes corresponding to portions thereof, can be used as a probe to screen DNA libraries and recover intact xylanase genes including the unique regions of the recovered DNA. Second, the recovered DNA or polynucleotides corresponding to portions thereof, can be inserted into a known xylanase gene to produce a recombinant xylanase gene with the sequence variations of the recovered DNA.

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METHOD FOR ISOLATING XYLANASE GENE SEQUENCES FROM
SOIL DNA, COMPOSITIONS USEFUL IN SUCH METHOD AND
COMPOSITIONS OBTAINED THEREBY

DESCRIPTION

Field of the Invention

This application relates to the use of PCR amplification to isolate novel xylanase genes from soil DNA, and to primers useful in such methods and the products obtained thereby.

Background of the Invention

The hydrolysis of cellulose, and hemicellulose, with xylans being a major component of hemicellulose, requires a variety of enzymes having activity as endoglucanases, exoglucanases, and xylanases to work in concert. It is with these systems of enzymes, composed of enzymes from the different cellulase families, that plant material is degraded in nature.

Cellulases have been classified into 12 families (designated A to L), and a single organism may have a set of enzymes with members drawn from several families. Of these families, families F and G show xylanase activity.

There has been an increasing awareness of the potential industrial uses for cellulases and xylanases; examples include biomass conversion, Saddler, J.N., *Bioconversion of forest and agricultural plant residues*, CAN International, Oxford, England (1993), and the role cellulases and xylanases are playing in pulp processing and paper production. Wick, C.B., *Genetic Engineering news* 14: 10-11 (1994). For example, xylanases can be used to make pulp bleaching more environmentally friendly by reducing organochlorine discharges. McCubbin, N., *Pulp & Paper Canada*, 95: 4 (1994).

In identifying and characterizing cellulases and xylanases suitable for use in industry, traditional methods of isolation and selection of cellulase and xylanase-producing organisms continues to be carried out by growth on cellulose and cellulose-like substrates. However, the traditional methods are only suitable for culturable organisms. Considering that it is estimated that only 1% of the organisms present in soil are culturable, Tiedje, J.M., *ASM News* 60:524-525 (1994), these traditional methods only skim the surface of the resource of enzymes which soil could theoretically provide.

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Bergquist et al., in a paper delivered at the Society for Industrial Microbiology Meeting in Montreal, Canada in June 1994 discussed methods for isolating hemicellulolytic enzymes from the extremely thermophilic bacteria in hot pools having temperatures as high as 95 C. For non-culturable organisms, they suggest that the polymerase chain reaction (PCR) on total DNA isolated from concentrated hot springs water with primers hybridizing to conserved regions of the known xylanase genes can be used to isolate xylanase DNA. Bergquist did not disclose or suggest methods for recovery of xylanase DNA from far more complex and challenging soil samples.

It is an object of the present invention to provide access to the cellulase and xylanase enzymes produced by non-culturable organisms by providing a mechanism for extracting DNA specific to Family F xylanases from soil.

It is a further object of this invention to provide specific compositions, particularly primers, useful in performing this isolation procedure.

It is still a further object of the invention to provide novel xylanase enzymes containing active sites which have been isolated from soil using the procedures of the present invention.

Summary of the Invention

The present invention provides a method for recovering xylanase-encoding DNA from soil, comprising the steps of:

- (a) treating a soil sample to render DNA in the soil accessible for hybridization with oligonucleotide primers;
- (b) combining the treated soil sample with first and second primers in an amplification reaction mixture, said first and second primers hybridizing with conserved regions of the sense and antisense strands respectively of a gene encoding a xylanase and flanking a region of interest in the gene;
- (c) thermally cycling the amplification reaction mixture through a plurality of cycles each including at least a denaturation phase and a primer extension phase to produce multiple copies of the region of interest flanked by the primers; and
- (d) recovering the copies of the region of interest from the amplification reaction mixture. Because of the complexity of the soil samples, it is likely that the recovered product will include more than one species of polynucleotide. Thus, these recovered copies

may, in accordance with the invention, be cloned into a host organism to produce additional copies of each individual species prior to characterization by sequencing.

Recovered DNA which is found to vary from known xylanases can be used in several ways to facilitate production of novel xylanases for industrial application. First, the recovered DNA, or probes corresponding to portions thereof, can be used as a probe to screen soil DNA libraries and recover intact xylanase genes including the unique regions of the recovered DNA. Second, the recovered DNA or polynucleotides corresponding to portions thereof, can be inserted into a known xylanase gene to produce a recombinant xylanase gene with the sequence variations of the recovered DNA.

Brief Description of the Drawings

Fig. 1 shows a map of a Family F xylanase gene showing the location of conserved regions suitable for use as primers; and

Fig. 2 shows the sequence differences between twenty DNA fragments isolated using the method of the invention and the sequence of the corresponding region of the Family F xylanase from *Cellulomonas fimi*.

Detailed Description of the Invention

Although the method of the invention for recovering xylanase DNA from soil samples appears in retrospect to be similar to PCR amplification of DNA from other sources (including the hot spring water of Bergquist et al.), the utility of PCR amplification in this environment and for this purpose could not be predicted with any confidence because of the complexity of soil. Soil is a complex mixture of minerals, decaying organic matter, and numerous organisms and microorganisms. As such it contains many possible sources of DNA, and many complex organic materials, e.g., humic materials, which could interfere with primer binding or polymerase enzyme activity to make PCR amplification unworkable. Thus, the very first question addressed in the development of the present invention was whether or not PCR amplification could be performed directly on a soil sample.

To determine whether PCR could be effectively used to amplify Family F cellulase gene fragments in the presence of humic substances carried over into extracted soil samples, soil DNA prepared by direct lysis as described in Barns, et al., *Proc. Natl. Acad. Sci.* 91: 1609-1613 (1994), was spiked with *Cellulomonas fimi* genomic DNA, and PCR was

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performed using degenerate primers hybridizing to conserved regions of Family F xylanase genes (Fig. 1) and processed in two rounds of PCR, for a total of 70 cycles. Agarose gel electrophoresis was used to separate the PCR products. Evaluation of these gels clearly showed two bands corresponding to about 300 and 400 base pairs for the spiked samples and for an undiluted genomic control. The lower band is the expected size (285bp) from *C. fimi* genomic DNA. The 400 bp band upon further investigation yielded a putative second family F cellulase member enzyme for *C. fimi*. With increasing dilution of the genomic DNA, more distinct PCR products appear in the regions outside of the 400 bp and 300 bp regions. Overall, these results indicate that the humic substances are not appreciably inhibiting the PCR, and PCR products could be obtained without optimization. In addition, at greater dilutions of the genomic DNA, the target sequences in the soil DNA experience less competition from the genomic DNA for primer binding. This leads to amplification of soil DNA targets.

Since the preliminary experiments showed that PCR could be used to amplify soil DNA, PCR was performed on unspiked soil DNA. In this case, PCR amplification resulted in the amplification of five bands greater than 300 bp. This result is not unexpected as the size of the fragments of family F cellulases that the constructed primers target, in known family F members, are quite heterogeneous, with variation between 195 bp and 345 bp, and further evaluation of the recovered fragments confirmed that the products are likely to be xylanase gene fragments based on homology to known genes. Thus, in accordance with the present invention there is provided a method for recovering xylanase DNA from soil, comprising the steps of:

- (a) treating a soil sample to render DNA in the soil accessible for hybridization with oligonucleotide primers;
- (b) combining the treated soil sample with first and second primers in an amplification reaction mixture, said first and second primers hybridizing with conserved regions of the sense and antisense strands respectively of a gene encoding a xylanase and flanking a region of interest in the gene;
- (c) thermally cycling the amplification reaction mixture through a plurality of cycles each including at least a denaturation phase and a primer extension phase to produce multiple copies of the region of interest flanked by the primers; and

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(d) recovering the copies of the region of interest from the amplification reaction mixture.

The soil sample employed in the present invention may be any type of soil that includes a mixture of mineral and organic materials. In the initial step of the method of the invention, a soil sample is treated to render the DNA accessible to the primers and enzymes employed in the amplification reaction. For example, DNA can be rendered accessible by a direct lysis procedure in which soil is treated with lysozyme, followed by Proteinase K, and then extracted with an organic solvent. DNA is precipitated from the aqueous phase and then further purified by chromatography. Incorporation of soil DNA into a phage library can also be performed, and such a library is a form of a treated soil sample within the scope of the present invention.

The treated soil sample is combined with two primers for PCR amplification in an amplification reaction mixture. The basic requirements for PCR amplification are well known, for example from US Patent No. 4,683,202 of Mullis, which is incorporated herein by reference and will not be described in detail. In general, however, the amplification reaction will include a thermostable polymerase enzyme such as Taq or Ultratherm™ polymerase and all four types of nucleotide triphosphates (A, C, G and T) in a buffer suitable for primer extension reactions.

The primers employed in the method of the invention can be any pair of primers which bind to conserved regions on complementary strands of a cellulase/xylanase gene and which flank a region of interest because of suspected structural diversity. Fig. 1 shows the location of the primers used by Bergquist et al. to amplify xylanase gene fragments from hot spring waters, which could be used to amplify soil DNA, and a preferred set of primers which produce larger fragments. These preferred primers are degenerate primers having the sequences
forward primer:

CGS GGS CAC ACS XTS XTS TGG [SEQ ID NO 1],

and reverse primer:

GTT GTA GTC GTT GWX GXA SA [SEQ ID NO 2],

where S indicates a C or G, W indicates an A or T, and X indicates an inosine.

The amplification reaction mixture containing the primers and the treated soil sample is subjected to a plurality of thermal cycles to produce amplified DNA fragments

corresponding to the region flanked by the primers. After thermal cycling, the amplification products are separated on an electrophoresis gel. Agarose gels have been found to be sufficient for this purpose, although polyacrylamide gels could also be used. Other separation techniques, including capillary electrophoresis and the use of biotinylated primers to facilitate capture of the amplified materials on an (strept)avidin-coated support might also be employed to recover the amplified DNA from the reaction mixture.

Because of the diversity of DNAs in soil samples, the products produced in the amplification reaction are likely to include more than one species of xylanase gene fragment. Thus, the recovered DNA is suitably cloned in a host organism to produce multiple copies of each species individually. We have used Invitrogen "Original TA cloning kit" that utilizes 3' A overhangs on the PCR product for ligation for cloning the amplified fragment into pCRII. This plasmid was then introduced into *E. coli* INV α F' by conventional means. The specific plasmid and host organism are not critical, however, and other plasmids and hosts could be also be used.

Plasmids containing the cloned soil DNA are recovered from the host organisms and evaluated by sequencing, preferably using a modification of the Sanger et al method. Sequencing primers that are the same as or similar to the original amplification primers can be used to obtain the sequence of the region flanked by the amplification primers, as can primers that hybridize with portions of the plasmid. Sequencing can be carried out using labeled primers or dye-labeled chain-terminating nucleotide triphosphates. The sequences determined are compared to known sequences for xylanase genes, for example using the BLAST program, to confirm that cloned fragment is indeed derived from a xylanase gene and to determine whether it has a previously uncharacterized sequence. Unique xylanase sequences are then further processed to obtain a complete gene of unique sequence for evaluation.

The process of obtaining a complete xylanase gene can be carried out in two ways. First, the recovered DNA, or selected portions thereof which contain unique base sequences can be used to select xylanase genes from a phage library containing soil DNA. While it will be understood that the specific techniques and reagents employed in the construction of a library of this type permit the exercise of a great many personal preferences, we constructed such a library from soil DNA prepared by a modification of the method described by Holben et al., *Appl. Environ Microbiol.* 53: 703-711 (1988). In this process, soil

samples are homogenized and the centrifuged at progressively greater g to isolate a bacterial pellet. The pellet is suspended in buffer, treated with Sarkosyl and then lysed with lysozyme. The lysed cells are treated with pronase followed by Sarkosyl. DNA was extracted from the supernatant of the resulting bacterial lysate by a standard phenol/chloroform extraction. The DNA was then precipitated by isopropanol. The DNA was further purified by centrifugation through Sephadex G-200 columns as follows.

The resulting soil DNA was partially digested (less than 20 minutes exposure to the enzyme) with 0.5 units of restriction endonuclease BstY I per ug of DNA and loaded on an 0.3% agarose gel from which 6 to 12 kilobase fragments were electroeluted. The ligation, packaging, and amplification protocols were followed as per Stratagene's Predigested ZAP Express *Bam*HI/CIAF Vector Cloning Kit, and the Gigapack III Gold Packaging Extract. The ligation was carried out with a 1 to 5 molar ratio of vector to insert DNA.

The resulting library is then screened to identify members of the library containing xylanase genes using probes based upon the novel sequences found from the initial amplification of soil DNA. The probe sequence may be the full length polynucleotide produced by amplification of the soil DNA and cloning. Alternatively, the probe sequence may be a polynucleotide which includes one or more of the unique genetic variations detected in the amplified products, in an otherwise known xylanase gene fragment. Probes used in this step may have lengths in the range of from 20 to 1500 bases, preferably 100 to 1000 bases.

Once identified, phagemids containing the selected xylanase inserts can be recovered and evaluated. The xylanase insert can, for example, be sequenced using primer walking over the inset to confirm the presence of the desired variation, or may be expressed and the expressed enzyme evaluated to determine the properties of the enzyme encoded by the insert.

As an alternative to the use of probes to isolate naturally occurring enzymes which deviate from the standard xylanase sequences, constructed xylanase genes can be formed using techniques such as site-directed mutagenesis or PCR-directed domain shuffling (See Cramer et al., *Nature Biotechnology* 14: 315-319 (1996), to introduce one or more sequence variations corresponding to variations found in amplified soil sample DNA. General techniques for introducing defined variations into known sequences are well known in the art, and so will not be repeated here.

Using the method of the invention, the present inventors have isolated and sequenced a total of twenty different xylanase DNA fragments that do not correspond to previously known xylanases and one complete novel xylanase gene. The sequences of these fragments and gene are given Seq. ID Nos. 3 - 22. Fig. 2 shows a comparison of the fragment sequences with the corresponding region of the xylanase from *C. fimi* (Seq. ID No. 23), with boxes drawn around regions containing regions of significant variability. Polynucleotides including one or more of these variations, and particularly polynucleotides including the boxed regions, can be utilized in designing probes or recombinant genes as discussed above.

The invention will now be further described with reference to the following non-limiting examples.

EXAMPLE 1

DNA was extracted from a soil sample using the "direct lysis" method as described in Barns et al., *Proc. Natl. Acad. Sci.* 91: 1609-1613 (1994). The resulting extracted soil sample was combined with two degenerate primers targeting highly conserved regions of family F cellulases, namely:

5'-CG(CG) GG(CG) CAC AC(CG) XT(CG) XT(CG) TGG-3' [Seq ID No 1]

and

5'-GTT GTA GTC GTT G(AT)X GXA (CG)A-3' [Seq ID No. 2]

where "X" indicates an inosine. Inosine was used to decrease the degeneracy of each primer. Patil, et al., *Nucleic Acid Res.* 18: 3080 (1990). These primers flank an active site of Family F cellulases such that variations in recovered sequences are likely to be significant to the function of the enzyme.

Amplification was performed on a MJResearch PTC-100 thermocycler as follows: 25-80 ng of template DNA, 0.50 ug of each primer, 50uM of each dNTP, 1.5mM of MgCl₂, 1X of 10X *Taq* buffer, and 5U of *Taq* polymerase (buffer and polymerase from GibcoBRL), were mixed with sterile distilled water to 50ul. Following a "hotstart" of 94°C for 3 min, cooling the mix in ice for 5 min, centrifuging, and maintaining at 80°C while loading the polymerase, a "touchdown" protocol was utilized to overcome the T_m difference of the primers and to prevent spurious priming. Don, et al., *Nucleic Acids Res.* 19: 4008 (1991); Roux, K.H., *BioTechniques* 16: 812-814 (1994). Thermocycling: denaturation, 94°C 50 sec; annealing, 65°C for 1 min; extension, 72°C for 1 min; and for the first 10 cycles,

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the annealing temperature was lowered 1°C per cycle until 55°C was reached. Then a subsequent 25 cycles were carried out with the annealing temperature at 55°C. A final extension for 10 min at 72°C was carried out. PCR products were analyzed by electrophoresis through a 1.5% agarose gel with ethidium bromide staining.

DNA was extracted from agarose gel by the QIAGEN Qiaex protocol, or by the "freeze-thaw" method involving the steps of: excision of the DNA band from the gel, freezing at -80°C for 20 min, thawing at 37°C for 10 min, the addition of 10 ul of H₂O, centrifugation at 15000 rpm in a minifuge for 2 min, then removing and saving the liquid. The extracted DNA was reamplified using the same primers, separated on an agarose gel and then cloned into pCRII plasmid using the Invitrogen "Original TA cloning kit." The plasmids were transformed into Invitrogen's competent *E. coli* cells.

Selection of cells containing transformed plasmids was performed by growth on LB media containing ampicillin and X-gal. White colonies were selected, and after overnight growth, cloned plasmids were purified using either QIAwell 8, or tip-20 modified alkaline lysis, and resin plasmid extraction and purification kits (from QIAGEN Inc.) and sequenced using an Applied Biosystems, Inc. PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit on an ABI 373 Stretch sequencer. Geneworks (by IntelliGenetics Inc.), Apple Mac version, was used for resolving sequence ambiguities, translation, and alignment construction. The determined DNA sequences were sent to the NCBI BLAST database located at, e-mail: blast@ncbi.nlm.nih.gov for the comparison of DNA sequences against protein databases.

Using this method, eight DNA fragments, denominated herein as Seq. ID. No. 3 through 10 were identified. Blast analysis confirmed the assignment of these fragments as derived from a xylanase gene, but did not produce an exact match for any of the fragments.

EXAMPLE 2

The experiment of example 1 was repeated except that different PCR reagents and conditions were used. In place of Taq polymerase, 1U of Ultratherm™ from BIO/CAN was used, and processed at a lower annealing temperature to see if this would generate a more diverse set of fragments. The thermocycling program used was: 94°C for 30 seconds; 45°C for 1 minute; increase temperature 1°C per 5 seconds to 72°C; 72°C for 45 seconds; repeat the previous steps 4 times, each time increasing the annealing temperature by 2°C; carry out

10 cycles of 94°C for 30 seconds, 53°C for 1 minute, 72°C for 45 seconds; then 94°C for 30 seconds, 55°C for 1 minute, increase temperature 1°C per 5 seconds to 72°C and 72°C for 45 seconds; then 30 cycles of 94°C for 30 seconds, 55°C for 1 minute, 72°C for 45 seconds; and a final extension step of 72°C for 10 minutes. This resulted in the recovery of an additional ten fragments denominated as Seq. ID Nos. 11 through 20 herein.

EXAMPLE 3

To prepare a phage library, soil DNA was first prepared by homogenizing a 50 g soil sample in a homogenization buffer containing 1.43 mM K_2HPO_4 , 1.01 mM $MgSO_4 \cdot 7H_2O$, 2.14 mM NaCl, 4.75 μM $Fe_2(SO_4)_3 \cdot 7H_2O$, 14.8 μM $MnSO_4 \cdot 4H_2O$ to which sodium ascorbate was added just before use to achieve a final concentration of 0.2 M. The homogenate was filtered through cheese cloth and the recovered solids suspended in 100 mL TE buffer to form a bacterial suspension. The suspension was brought to 1 M NaCl by addition of 25 mL of 5 M NaCl, incubated at room temperature for 10 minutes and then collected by centrifugation. The pellet was resuspended in TS buffer (50 mM Tris, pH 8.0; 50 mM NaCl) transferred to a 50 mL polycarbonate centrifuge tube and brought to a concentration of 0.1% Sarkosyl by addition of 50 μL of 20% Sarkosyl. This mixture was incubated at room temperature for 10 minutes, after which the bacteria were collected by centrifugation. The bacterial pellet was drained and suspended in 35 mL of Tris-sucrose-EDTA which contains 0.75 M sucrose, 50 mM Tris (pH 8.0) and 10 mM EDTA. Lysozyme was added to a final concentration of 5 mg/ml and the samples were incubated at 37°C for 60 minutes. A pronase solution in TS buffer that had been predigested by incubation for 30 minutes at 37°C was added to the bacteria-lysozyme mixture, mixed by vortexing, and then incubated at 37°C for 60 minutes. The temperature was then raised to 65°C and 0.25 ml 20% Sarkosyl was added and incubated for 10 minutes. DNA was extracted from the supernatant of the resulting bacterial lysate by a standard phenol/chloroform extraction. The DNA was then precipitated by isopropanol. The DNA was further purified by centrifugation through Sephadex G-200 columns as follows.

2 grams of Sephadex G-200 (Pharmacia Biotech) were washed 5 times with 75 ml TE Buffer pH 8.0 (10mM Tris-HCl, 1 mM EDTA). Each time, the mixture was allowed to settle and excess TE drawn off before adding more TE. Then the Sephadex suspension was autoclaved. Excess TE was drawn off and the suspension brought to the original volume

with high salt TE buffer pH 8.0 (10mM Tris-HCl, 1mM EDTA, 0.1M NaCl), shaken and allowed to settle. Excess TE was drawn off and the suspension was again brought to the original volume with high salt TE buffer, and shaken again. A 5ml syringe was packed with sterile fiberglass to the 1cc mark, and Sephadex added. This column was then spun in a swing-bucket centrifuge for 10 minutes at 1000 x g in a sterile test tube, 500 ul of the high-salt TE was added, and the column was spun again for 10 minutes at 1000 x g. The column was then transferred to a new test tube, the DNA added to the column, and spun for 10 minutes at 1000 x g. For three more times, 500 ul of the high-salt TE was added and the column spun for 10 minutes at 1000 x g. A final dry spin for 10 minutes at 1000 x g was carried out. The DNA was then precipitated with 1/10 volume of 3M Sodium Acetate and two volumes of 95 % Ethanol. The suspension was held over night at 4°C. This was then centrifuged for 20 minutes in a minifuge at 4°C, the supernatant was removed and replaced with 70 % Ethanol and re-centrifuged. The supernatant was removed and the pellet was dried, and dissolved in TE (not high-salt).

The resulting soil DNA preparation was partially digested (less than 20 minutes exposure to the enzyme) with 0.5 Units of *Bsr*YI per ug of DNA and 6 to 12 kilobase fragments were electroeluted from 0.3% agarose gel. The ligation, packaging, and amplification protocols were followed as per Stratagene's Predigested ZAP Express *Bam*HI/CIAP Vector Cloning Kit, and the Gigapack III Gold Packaging Extract. The ligation was carried out with a 1 to 5 molar ratio of vector to insert DNA.

Although probes having sequences derived from any of Seq ID Nos. 3 to 20 could have been used to screen the library, we chose to prepare additional probes by PCR amplification of the library stock. 5 ul of a 1.1×10^5 pfu/ul library stock, 50 uM final concentration of each dNTP, 0.5 uM final concentration of each degenerate primer (Seq. ID Nos. 1 and 2), 1.5mM final concentration of $MgCl_2$, 10% DMSO, 1X of 10X Ultratherm buffer, 1U of Ultratherm polymerase (buffer and polymerase from BIO/CAN Scientific, Ontario, Canada), and sterile, distilled water were mixed. Thermocycling: 94°C for 50 seconds; 65°C for 1 minute; 72°C for 1 minute; and for the first 10 cycles, the annealing temperature was lowered 1°C per cycle until 55°C was reached. A subsequent 35 cycles were carried out with the annealing temperature at 55°C, then a final extension for 10 minutes at 72°C. The Invitrogen "Original TA cloning kit" was used for cloning as in Example 1. Extra ATP was added to a final concentration of 1mM. Plasmid DNA was extracted and purified

with QIAGEN's tip-20 kit. The probe was prepared by digesting the TA vector with insert, with *EcoRI*. The digested sample was electrophoresed through a 1.2% agarose gel with ethidium bromide staining. The band of interest was cut out of the gel and the DNA fragment purified using QIAGEN's QIAEX kit. This procedure led to the identification of two additional xylanase fragments, denominated herein as Seq. ID Nos. 21 and 22. The fragment was labeled using GibcoBRL's Random Primers DNA Labeling System with [α - 32 P]dCTP as per provided protocol.

EXAMPLE 4

Screening of the library was performed using the fragment with the sequence given by Seq. ID. No. 21 as a probe. The screening protocol supplied with Stratagene's Pre-digested ZAP Express *Bam*HI/CIAP Vector Cloning Kit was followed. The post-hybridization washes were as follows: two washes in 0.5X SSC, 0.1% (w/v) SDS at 55°C; followed by one 0.5X SSC, 0.1% (w/v) SDS wash at 60°C. Next, Stratagene's recommended *in vivo* excision protocol was followed to isolate *E. coli* colonies containing the pBK-CMV phagemid with insert DNA. Phagemid DNA with insert was extracted and purified with QIAGEN's tip-20 kit.

EXAMPLE 5

A xylanase gene contained in a phagemid from the library was sequenced by primer walking over the insert using the degenerate amplification primers (Seq. ID Nos. 1 and 2) as initial extension primers. Then, subsequent extension primers were constructed by looking at the previously-generated sequence data. The sequence of the xylanase gene and putative amino acid sequence of the encoded-xylanase are set forth herein as Seq. ID No. 24.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Terragen Diversity Inc.
Radomski, Christopher C. A.
Seow, Kah Tong
Warren, R. Antony J.
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(ii) TITLE OF INVENTION: METHOD FOR ISOLATING XYLANASE GENE
SEQUENCES FROM SOIL DNA, COMPOSITIONS USEFUL IN SUCH METHOD AND
COMPOSITIONS OBTAINED THEREBY

(iii) NUMBER OF SEQUENCES: 24

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 Mb

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS 5.0

(D) SOFTWARE: Word Perfect

(vi) CURRENT APPLICATION DATA :

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION :

(A) NAME: Eileen McMahon

(B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (416)-941-9027

(B) TELEFAX: (416)-941-9443

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

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(A) NAME/KEY: degenerate primer for amplification of xylanase fragments from soil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
CGSGGSCACA CSNTSNTSTG G 21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: degenerate primer for amplification of xylanase fragments from soil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GTTGTAGTCG TTGWNGNASA 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGGCCACAC GGTCGTGTGG GCGGTGGACG ACTTTGTGCA GTCATGGATC	50
AAAAACCTTT CCAACGGGGA CCTGCGGATC CATTGACCA ACCGCATCGA	100
AAGCGTAGTC ATTCATTTCA CGGGCACCTT CATGCATCGG GATGTGAACA	150
ACGAAATGTT GCACGGCAAT TACTACGGCA ACCGCCTCGG CGATTCCATC	200
AACTCCTGGA TGTTCAAACA CGCCCGCTTG CAGGACAGCA ACGTCGTGCT	250
CTCCCTCAAC GACTACAAC	269

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: genomic DNA
 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: no
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM:
 (ix) FEATURE:
 (A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGCGGGGCACA CCGTCGTGTG GCACAACCAG CTTCCCGGGT GGGTGACGGC	50
GACGGCCCGC AGCAGCGACG AGCAGGCCGC GGTGCTGCAG GCGCACGTCA	100
CTCAGGAGGT CGACCACTTC CGCGGCCACA TCTACGCGTG GGACGTCGTC	150
AACGAGCCGT TCAACGATGA CGGCACCTGG CGCGACACCA TCTGGTACCG	200
CCCCATGGGT CCGGACTACA TCGCGCAGGC CTTCCGCTGG GTCCGCGCGG	250
CGGACCTAGA TGCCCGGCTG TCCCACAACG ACTACAAC	288

(2) INFORMATION FOR SEQ ID NO:5:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 288
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: genomic DNA
 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: no
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM:
 (ix) FEATURE:
 (A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGGGGGCACA CCGTGGTGTG GCACAACCAG CTTCCCGGGT GGGTGACGGC	50
GACGGCCCGC AGCAGCGACG AGCAGGCCGC GGTGCTGCAG GCGCACGTCA	100
CTCAGGAGGT CGACCACTTC CGCGGCCACA TCTACGCGTG GGACGTCGTC	150
AACGAGCCGT TCAACGATGA CGGCACCTGG CGCGACACCA TCTGGTACCG	200
CGCCATGGGT CCGGACTACA TCGCGCAGGC CTTCCGCTGG GCTCGCGCGG	250
CGGACCTAGA TGCCCGGCTG TCCCTCAACG ACTACAAC	288

(2) INFORMATION FOR SEQ ID NO:6:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 288
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: genomic DNA
 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: no
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM:

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(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGTGGGCACA CCGTCGTGTG GCACAACCAG CTGCCCCGGCT GGGTCACCAC	50
CGGTGCCTTC AGCAGCGACG AGCTCGCCGT CATCCTGCAG CAGCACATCA	100
CCGAGAAGGT CGGACACTTC GCCGGGCACA TCTCCGTGTG GGACGTGGTC	150
ATCGAGCCGC TCAACGACGA TGGCACCTGG CGCGACACCA TCTGGTACCG	200
CGCTCTGGGT CCGGGTTACG TCACGCAGGC GTTGCCTGG GCGCACGCGG	250
CTGACCCCGG CGCCAGGCTG TCCCTCAACG ACTACAAC	288

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCACAACCA GTTGCCAGCC TGGCTCACAA GCGGTGCATT CAGCAGCGCC	50
GAGCTGGCCA CCATCCTGGA GCAGCAGTC ACCCAGGAAG CGGACCATT	100
CCGCGGGCAC ATCTACGCCT GGGACATCGT CAACGAGCCG TTCAACGACG	150
ATGGCACCTG GCGTGACAGC CTCTGGTACC GCGCGCTGGG CGCCGGCTAC	200
GTCGCCCAGG CGTTGCGCTG GGCCCGCGCG GCCGATCCGT CTGCCCAGTT	250
CTCCCTCAAC GACTACAAC	269

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 282

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCGGGCACA CCGTCGTCTG GCACTCGCAA CTGCCGTCGT GGGTCAGTAA	50
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TCTTCCGACC AACCAGGTGC AGTCGGTGAT GGAAGCCCAC ATCACGACCG	100
AGGCCACCCA CTACAAGGGG AAGGTCTACG CCTGGGACGT CGTCAATGAA	150
CCGTCCAACG ACGACGGTAC GCTGCGCCAG GAGGTTTCT ATCGTGCCAT	200
GGGCACCGGC TACATCGCCG ACGCGATCCG TACCGCCCAC ACCGCCGACC	250
CCACCGCCAA GCTCTCCCAC AACGACTACA AC	282

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 282

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGGGGCACA CGGTCGTCTG GCACTCGCAA CTGCCGTCGT GGGTCAGTAA	50
TCTCCCGACC AACCAGGTGC AGTCGGTGAT GGAAGCCCAC ATCACGACCG	100
AGGCCACCCA CTACAAGGGG AAGGTCTACG CCTGAGACGT CGTCAATGAA	150
CCGTTCAACG ACGACGGTAC GCTGCGCCAG GACGTTTTCT ATCGTGCCAT	200
GGGCACCGGC TACATCGCCG ACGCGATCCG TACCGCCCAC ACCGCCGACC	250
CCACCGCCAA GCTCTCCCTC AACGACTACA AC	282

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGGGGGCACA CCGTCGTGTG GCACTCGCAG CTCTCCACCT GGCTGACGTC	50
GGGCACGTGG ACCGCCGCGC AGGCGACGAC GCTGATGACG GACCACATCG	100
CCAACGTCGT CGGCCACTAC AAGGGGCGAG TCGTCGGGTG GGACGTGGTC	150

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AACGAAGCGC TGAACGACGA TGGCACGTAT CGGTCTGGGGT TCTACTACGA	200
CCACATCGGC CCGACGTACA TCGAGACGGC GTTCCGCGCG GCGCACACCG	250
CCGACTCGAC GGTGCTGCTG TCCCACAACG ACTACAAC	288

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 291

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGCGGGCACA CCGTCGTCTG GCACGACCAG CTCTCCACCT GGGTGACGAC	50
GGGCAATTAC AGCGCTGCCC AAGCGGACTC CATTCTCGTA TCGTACATCA	100
CCACTGTGAT GACGCGATAC AAGGGTAAGG TCGGGATCTG GGATGTCGTC	150
AATGAAGCCA TGGGCGATGA TGCAGTGATC CGCACCTCGT CCTATTGGTA	200
TCAGAAGCTC GGACCGAACT ACATCGAGCG CGCATTTCGT CTCGCCAACA	250
GCGTTGATCC GACGGCAAAG CTGTCCCTCA ACGACTACAA C	291

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 298

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGCCACACG GTGGTCTGGC ATAACCAGAC GCCCAAGTGG GTCTTCAAG	50
ACGACAAGGG TCAACCCCTC ACTCGCGACG CCTCCTCGT CCGTCTCAA	100
GAGCACATTA ATAAGGTAGT CGGCCGCTAC AAAGGCCGTA TCAACGGTTG	150
GGACGTCGTC AACGAGGCCA TCAACGAAGA CGGCACCATG CGCCAGTCGC	200
CCTGGATGAA GATCATCGGC GACGACTTCA TCGAACTCGC ATTCCAGTAC	250

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GCGCACGACG CCGACCCGCA AGCCGAGCTC TCCCACAACG ACTACAAC

298

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 282

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGGCACACCG TGGTCTGGCA CTCGCAACAG CCAGGCTGGA TGCAGAGCCT	50
GAGCGGCACC GCCCTGCGCA ACGCCATGAT CAACCATATC AACGGCGTGA	100
TGGCCCACTA TAAAGGCAAG CTCGCCTACT GGGATGTGGT CAACGAAGCC	150
TTCGCGGACG ACGGCAGCCA GAACCGCCGC AACTCGAACC TCCAGCAGAC	200
CGGCAACGAC TGGATCGAGG TCGCCTTCAA GACGGCTCGC GCCGCCGATG	250
GCTCGGTCAA GCTCTCCAC AACGACTACA AC	282

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 311

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCACACGGT GGTCTGGCAT TCGCAGACGG GCGGCTGGTT CTTCCAGGGC	50
GCCGATGGTC AGCCGGCGAC GCGCGAAGTA GTGATGGAGC GGCTCCATAA	100
GCACATCACG ACGGTCGTCG GCCGCTACAA AGGAAAGGTC CTTGGGTGGG	150
ACGTCGTCAA TGAGTCGATC AACGACAATG GCGACGGCAC GACGGAAAAC	200
CTGCGGACGA GCAGTTGGTA TCGTGCGATC GGGCCGGATG TGCTGACGAT	250
GGCGTTCAAG TGGGCGCATG AAGCGGATCC GGATGCGCTG CTCTCCCTCA	300
ACGACTACAA C	311

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(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 300
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGGGGGCACA CGGTGGTCTG GCATAACCAG ACGCCCAAGT GGGTCTTCGA	50
AGACGACAAG GGTCAACCCC TCACTCGCGA CGCCCTCCTC GTCCGTCTCA	100
AAGAGCATAT TAATAAGGTA GTCGGCCGCT ACAAAGGCCG TATCAACGGT	150
TGGGACGTCG TCAACGAAGC CATCAACGAA GACGGCACCA TGCGCCAGTC	200
GCCCTGGATG AAGATCATCG GCGACGACTT CATCGAACTC GCATTCCAGT	250
ACGCGCACGA CGCCGACCCG CAAGCCGAGC TCTCCCAACG CGACTACAAC	300

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 288
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGGGGCCACA CCGTCGTCTG GCAGAACCAG CTGCCGGACT GGCTGACCAC	50
CGGCACCTAC ACGTCGGCAC AGCTGCGAGA CCTGTTGCAC AGGCACATCA	100
CCGACGAGGT CTCGCACTTC AAGGGTCACA TCTGGCAGTG GGATGTCGTC	150
AACGAGGCGT TCAACGACGA CGGCACGATG CGGGACACCC TCTGGCTGCG	200
CGCCATGGGC CCTGGGTATG TTGCCGACGC GTTCCGCTGG GCTCACCAGG	250
CAGATCCGGG TGCCCTGCTC TCCCTCAACG ACTACAAC	288

(2) INFORMATION FOR SEQ ID NO:17:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGCGGGCACA CCGTGGTGTG GCATCAGTGT GTGCCGGATT GGTAGCGAA	50
TGGAAATTTT ACTCGCGATG AGGCAATCGA ACTGTTGCAC AATCATATCT	100
CGACCGTGAT GGGACACTAC AAGGGGCGCA TCCTTGACTG GGATGTGGTC	150
AATGAAGCGA TTGCTGATAG TACTCTGCTG CGCGATACGC CCTGGCGAAA	200
ATTCATCGGC GACGACTATA TTGAAATGGC CTTTCGCTTC GCCCACGAAG	250
CCGATCCAGA TGCCTCCTC TCCCTCAACG ACTACAAC	288

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 282

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGGGGGCACA CCGTGGTGTG GCACAAGCAA CTGGGCGGCT GGGTCGAACA	50
ACTGGACGCG CCCGCGTTGC GAGCCGCGCT CGAGCACCAC ATTCGAACCG	100
TCGTGGGGCA CTACAAGGGG AAACCTCTGG CCTGGGACGT CGTCAACGAG	150
GCCCTGGGCG ACGACGGCAG CCCTCGCAAG ACGGTCTTCC TGAAAAGCT	200
GGGTCCCGGA TACATCGCCG ATGCGTTCCG CTGGGCGCAT GAGGCCGATC	250
CCCAGGCTCT GTTGTCCCTC AACGACTACA AC	282

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 300
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGGGGGCACA CGGTGGTCTG GCATAACCAG ACGCCCAAGT GGGTCTTCGA	50
AGACGACAAG GGTCAACCCC TACTCGCGA CGCCCTCCTC GTCCGTCTCA	100
AAGAGCACAT TAATAAGGTA GTCGGCCGCT ACAAAGGCCG TATCAACGGT	150
TGGGACGTCG TCAACGAAGC CATCAACGAA GACGGCACCA TCGCCAGTC	200
GCTCTGGATG AAGATCATCG GCGACGACTT CATCGAACTC GCATTCCAGT	250
ACGCGCACGA CGCCGACCCG CAAGCCGAGC TCTCCCACAA CGACTACAAC	300

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 296

(B) TYPE: nucleic acid

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGGGGCACAC GGTGGTGTGG CATCAACAGA ACCCAGCGTG GTTAACGGGC	50
ACTACGTGGA ACGTTGACAC ACTCAAGCTA CTGCTCAAGG AACACGTTGA	100
CAGCGTGGTC GGGCATTTC AAGGCAAGAT TCGCCGCGTGG GATGTCGTAA	150
ACGAAGCGTT CAACGATGGC ACGGGTACAC TTCGAACAAC GGATTCTCCG	200
TGGGCCACAA CCATTGGGCG TTCGTACGTT GAACTCGCGT TCAGAGAAGC	250
ACGCGCCATC GATCCGGCCG CGCAGCTGTC CCACAACGAC TACAAC	296

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 282
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM:
- (ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGGGGCCACA CGGTGGTCTG GCAGAACCAG CTACCGTCCT GGGTGTCCAG	50
CCTGCCGCTG AACCAGGTGC AGCAGGCGAT GGAAAGCCAC ATCACCACGG	100
AGGCCAGCCA CTACAAGGGC CAGGTTTACG CCTGGGACGT CGTCAACGAG	150
CCGTTCAACG GCGACGGCAG CTTCGTCAGC GATGTGTTTT ACCGTGCGAT	200
GGGCAGCGGG TACATCGCCG ACGCGCTGCG CACCGCGCAC GCCGCCGACC	250
CCGGCGCTCA GCTGTCCCTC AACGACTACA AC	282

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 294
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM:
- (ix) FEATURE:

(A) NAME/KEY: fragment of xylanase gene from degenerate primer amplification of soil DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGGGGCACA CCGTGGTGTG GTACGCGCAG AAGCCGGCGT CGTTCGAGCG	50
CCTGGTCAGC GACGCCGGCG CGTTTCGCGA CGCCTACGCC GCCTACATCA	100
CGGCCGTGGT TGGCCGCTAC AGGGGGCCGA TCGCCGGCTG GGGCGTCGTC	150
AACGAGCAGG TGACCGACGA CGGCGCCGCG TGGCGGGACA GCCTCTGGAG	200
CCACGCGCTC GGACCGCTGG AACACATGCG CTTCGCCTAT GAACTGGCCC	250
ACGCCGCCGA CCGCGGGCC GACCTGTCCC TCAACGACTA CAAC	294

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 285
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Cellulomonas fimi
- (ix) FEATURE:

(A) NAME/KEY: sequence of internal fragments of xylanase gene
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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TACGGCCACA CGCTCGTATG GCACTCGCAG CTGCCCCGACT GGGCGAAGAA   50
CCTCAACGGC TCCGCGTTTCG AGAGCGCGAT GGTCAACCAC GTGACGAAGG   100
TCGCCGACCA CTTTCGAGGGC AAGGTCGCGT CGTGGGACGT CGTCAACGAG   150
GCGTTCCGCG ACGGCGGCGG CCGCCGGCAG GACTCGGCGT TCCAGCAGAA   200
GCTCGGCAAC GGCTACATCG AGACCGCGTT CCGGGCGGCA CGTGCGGCGG   250
ACCCGACCGC CAAGCTGTGC ATCAACGACT ACAAC                       285
  
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1524
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM:
- (ix) FEATURE:

(A) NAME/KEY: sequence of xylanase gene identified by
 amplification of xylanase fragments from soil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```

ATG ACC GTG AGA TCA ATC CAG AAG AGG CTT CGC GTA TCG CGG CGC 45
Met Thr Val Arg Ser Ile Gln Lys Arg Leu Arg Val Ser Arg Arg

GGC GGT GGC GCC CGC GCC GGC CGG CCA CGT CAA CAG GTG CTG ACA 90
Gly Gly Gly Ala Arg Ala Gly Arg Pro Arg Gln Gln Val Leu Thr

GCG GTG GCG GCG ACT GCC TGC GTC GCG GGC GGC GCG CTC GCC GCG 135
Ala Val Ala Ala Thr Ala Cys Val Ala Gly Gly Ala Leu Ala Ala

GCA GTG CTG GCC GCG GCC GGG CCG GCC ACG GCG GCC GGC AGC ACG 180
Ala Val Leu Ala Ala Ala Gly Pro Ala Thr Ala Ala Gly Ser Thr
  
```

- 25 -

CTG CCG GCG GCG GCT GAG GCG CAG GGC AAG TAC TTC GGG ACT GAG 225
Leu Arg Ala Ala Ala Glu Ala Gln Gly Lys Tyr Phe Gly Thr Glu

GTC ACC GGG AAC ATG ATC AAC AAC TCG ACG ATC ACG AAC CTG GCA 270
Val Thr Gly Asn Met Ile Asn Asn Ser Thr Ile Thr Asn Leu Ala

GGC CAG CAG TTC GAC ATG GTC ACC CCG GGC AAC GAG ATG AAG TGG 315
Gly Gln Gln Phe Asp Met Val Thr Pro Gly Asn Glu Met Lys Trp

GAC ACC ACC GAG CCG TCC AAC GGG TCC TAC AAC TTC GGC CCG GGC 360
Asp Thr Thr Glu Pro Ser Asn Gly Ser Tyr Asn Phe Gly Pro Gly

GAC GCG GTC GTG TCG TTC GCC AAG GCG CAC GGC ATG CCG GTG CGC 405
Asp Ala Val Val Ser Phe Ala Lys Ala His Gly Met Arg Val Arg

GGG CAC AAC CTG GTC TGG CAG AAC CAG CTC CCG TCG TGG GTT TCC 450
Gly His Asn Leu Val Trp Gln Asn Gln Leu Pro Ser Trp Val Ser

AGC CTG CCG CTG AAC CAG GTG CAG CAG GCG ATG GAA AGC CAT GTC 495
Ser Leu Pro Leu Asn Gln Val Gln Gln Ala Met Glu Ser His Val

ACC ACG GAG GCC AGC CAC TAC AAG GGC CAG GTT TAC GCC TGG GAC 540
Thr Thr Glu Ala Ser His Tyr Lys Gly Gln Val Tyr Ala Trp Asp

GTC GTC AAC GAG CCG TTC AAC GGC GAC GGC AGC TTC GTC AGC GAC 585
Val Val Asn Glu Pro Phe Asn Gly Asp Gly Ser Phe Val Ser Asp

GTG TTT TAC CGC GCG ATG GGC AGC GGG TAC ATC GCC GAC GCG CTG 630
Val Phe Tyr Arg Ala Met Gly Ser Gly Tyr Ile Ala Asp Ala Leu

CGC ACC GCG CAC GCC GCC GAC CCC AGT GCC CAG CTG TAC ATC AAC 675
Arg Thr Ala His Ala Ala Asp Pro Ser Ala Gln Leu Tyr Ile Asn

GAC TAC AGC ATC GAG GGC GAG AAC GCC AAG AGC AAC GCC ATG TAC 720
Asp Tyr Ser Ile Glu Gly Glu Asn Ala Lys Ser Asn Ala Met Tyr

AGC CTG GTG CAG TCC CTG CTG GCG CAG GGG GTG CCG ATC AAC GGC 765
Ser Leu Val Gln Ser Leu Leu Ala Gln Gly Val Pro Ile Asn Gly

GTG GGC TTT GAA AGC CAC TAC ATC GTG GGG CAG GTG CCC TCG TCG 810
Val Gly Phe Glu Ser His Tyr Ile Val Gly Gln Val Pro Ser Ser

CTG CTG GCC AAC ATG CAG CCG TTC GCT GCC CTG GGC GTC AAC GTG 855
Leu Leu Ala Asn Met Gln Arg Phe Ala Ala Leu Gly Val Asn Val

GCG GTC ACC GAG CTT GAC GAC CGC GTC CAG CTG CCG GCC AGC ACC 900
Ala Val Thr Glu Leu Asp Asp Arg Val Gln Leu Pro Ala Ser Thr

GCG AGC CTG AAC CAG CAG GCC ACC GAC TAC GCC ACC GTG GTG CGC 945
Ala Ser Leu Asn Gln Gln Ala Thr Asp Tyr Ala Thr Val Val Arg

- 26 -

GAC TGC CTG CAG GTC TCC CGC TGC GTC GGC GTG TCG CAA TGG GGC 990
Asp Cys Leu Gln Val Ser Arg Cys Val Gly Val Ser Gln Trp Gly

GTC GGC GAC GCC GAC TCC TGG ATC CCG GGA ACG TTC CCC GGC TGG 1035
Val Gly Asp Ala Asp Ser Trp Ile Pro Gly Thr Phe Pro Gly Trp

GGC GCG GCG ACC ATG TAC GAC CAG AAC TAC CAG CCC AAG CCC GCG 1080
Gly Ala Ala Thr Met Tyr Asp Gln Asn Tyr Gln Pro Lys Pro Ala

TAC TCC GCC ACC TTG TCC GCC CTC GGC GGC TCC GGC AGC ACC GGC 1125
Tyr Ser Ala Thr Leu Ser Ala Leu Gly Gly Ser Gly Ser Thr Gly

GGT GGC AGC GGC GAG ATC CAC GCG GTC GGG GCG GGC AAG TGC CTG 1170
Gly Gly Ser Gly Glu Ile His Ala Val Gly Ala Gly Lys Cys Leu

GAC GTG CCC GGC CTC GCC ACC ACC GCG GGC ACC CAG CTG GAC ATC 1215
Asp Val Pro Gly Leu Ala Thr Thr Ala Gly Thr Gln Leu Asp Ile

TGG ACC TGC AAC GGC GGC ACC AAC CAG ATC TGG ACG CAC ACC TCC 1260
Trp Thr Cys Asn Gly Gly Thr Asn Gln Ile Trp Thr His Thr Ser

GCC AAC CAG CTG ACC GTC TAC AGC GGC AGC AGC CAG ATG TGC CTG 1305
Ala Asn Gln Leu Thr Val Tyr Ser Gly Ser Ser Gln Met Cys Leu

GAC GCT TAC AAC AAC CAG ACC ACC CCC GGC ACC AAG GTG GAC ATC 1350
Asp Ala Tyr Asn Asn Gln Thr Thr Pro Gly Thr Lys Val Asp Ile

TGG ACG TGC AAC GGC GGC GCT AAC CAG CAG TGG CAC GTC AAC TCC 1395
Trp Thr Cys Asn Gly Gly Ala Asn Gln Gln Trp His Val Asn Ser

AAC GGC ACG ATC ACC AGT GCC CAG TCC GGG CTG TGC CTG GAC GTG 1440
Asn Gly Thr Ile Thr Ser Ala Gln Ser Gly Leu Cys Leu Asp Val

ACC GGC GCC AGC ACC GCC AAC GGC GCG CTG GCC GAG CTG TGG ACC 1485
Thr Gly Ala Ser Thr Ala Asn Gly Ala Leu Ala Glu Leu Trp Thr

TGC AAC AGC CAG TCC AAC CAG CAA TGG ACC CTC GGA TGA 1524
Cys Asn Ser Gln Ser Asn Gln Gln Trp Thr Leu Gly ***

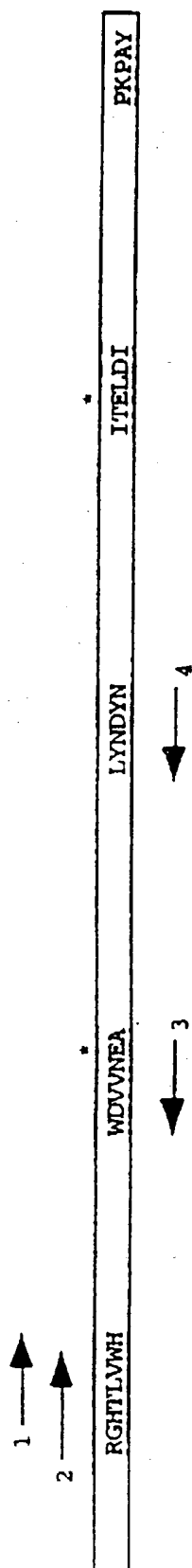
- 27 -

CLAIMS

1. A method for recovering xylanase DNA from soil, comprising the steps of:
 - (a) treating a soil sample to render DNA in the soil accessible for hybridization with oligonucleotide primers;
 - (b) combining the treated soil sample with first and second amplification primers in an amplification reaction mixture, said first and second amplification primers hybridizing with conserved regions of the sense and antisense strands respectively of a gene encoding a xylanase and flanking a region of interest in the gene;
 - (d) thermally cycling the amplification reaction mixture through a plurality of cycles each including at least a denaturation phase and a primer extension phase to produce multiple copies of the region of interest flanked by the first and second amplification primers; and
 - (e) recovering the copies of the region of interest from the amplification reaction mixture.
2. The method of claim 1, wherein the first and second amplification primers include at least one primer having the sequence given by Seq. ID. No. 1 or 2.
3. The method according to claim 1 or 2, further comprising the step of determining the nucleotide sequence of the recovered copies.
4. The method according to claim 3, wherein the sequence of the recovered copies is determined by inserting the recovered copies into plasmids having an origin of replication, transforming a bacterial host with the modified plasmids and evaluating the sequence of the insert within plasmid produced by a transformed bacterial host.
5. The method according to any of claims 1 to 4, further comprising the step of screening a treated soil sample to isolate full length DNA using a probe having a sequence which is the same as or complementary to at least a portion of a recovered copy of the region of interest, said portion being different from the reference xylanase sequence given by Seq. ID. No. 23.
6. The method according to claim 5, wherein the probe has a sequence which is the same as or complementary to at least a portion of any one of Seq. ID Nos. 3 through 22 that is different from the reference xylanase sequence given by Seq. ID. No. 23.

7. The method according to claim 5, wherein the probe has a sequence which is the same as or complementary to any one of Seq. ID Nos. 3 through 22.
8. A xylanase DNA fragment recovered from soil by the methods of any of claims 1 through 7.
9. A method for recovering a xylanase gene from soil, comprising the steps of:
 - (a) combining a treated soil sample in which soil DNA is rendered accessible for hybridization with a polynucleotide probe having a sequence which is the same as or complementary to at least a portion of any one of Seq. ID Nos. 3 through 22 that is different from the reference xylanase sequence given by Seq. ID. No. 23; and
 - (b) isolating DNA that hybridizes with the probe from the treated soil sample.
10. The method according to claim 9, wherein the treated soil sample is a phage library prepared from soil sample.
11. A substantially purified xylanase gene isolated by the methods of any of claims 9 or 10.
12. A substantially purified xylanase gene having the sequence given by Seq. ID No. 24.
13. A recombinant xylanase gene comprising a standard region and a modified region, said standard region having a sequence which corresponds to a known xylanase sequence given by Seq. ID No. 23, and said modified region having a sequence which is the same as or complementary to at least a portion of any one of Seq. ID Nos. 3 through 22 that is different from the reference xylanase sequence given by Seq. ID. No. 23.
14. A polynucleotide probe for isolation or identification of xylanase genes having a sequence which is the same as or complementary to at least a portion of any one of Seq. ID Nos. 3 through 22 that is different from the reference xylanase sequence given by Seq. ID. No. 23.
15. A polynucleotide probe for isolation or identification of xylanase genes having a sequence which is the same as or complementary to any one of Seq. ID Nos. 3 through 22.

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1. Forward primer from Bergquist et al.
2. Forward primer from the present invention.
3. Reverse primer from Bergquist et al.
4. Reverse primer from the present invention.

Fig. 1

1	18	107	134	269
2	19	108	211	296
3	20	109	212	311
4	21	110	213	315
5	22	111	214	318
6	23	112	215	322
7	24	113	216	324
8	25	114	217	328
9	26	115	218	332
10	27	116	219	336
11	28	117	220	338
12	29	118	221	342
13	30	119	222	346
14	31	120	223	348
15	32	121	224	352
16	33	122	225	356
17	34	123	226	358
18	35	124	227	362
19	36	125	228	366
20	37	126	229	368
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22	39	128	231	376
23	40	129	232	378
24	41	130	233	382
25	42	131	234	386
26	43	132	235	388
27	44	133	236	392
28	45	134	237	396
29	46	135	238	398
30	47	136	239	402
31	48	137	240	406
32	49	138	241	408
33	50	139	242	412
34	51	140	243	416
35	52	141	244	418
36	53	142	245	422
37	54	143	246	426
38	55	144	247	428
39	56	145	248	432
40	57	146	249	436
41	58	147	250	438
42	59	148	251	442
43	60	149	252	446
44	61	150	253	448
45	62	151	254	452
46	63	152	255	456
47	64	153	256	458
48	65	154	257	462
49	66	155	258	466
50	67	156	259	468
51	68	157	260	472
52	69	158	261	476
53	70	159	262	478
54	71	160	263	482
55	72	161	264	486
56	73	162	265	488
57	74	163	266	492
58	75	164	267	496
59	76	165	268	498
60	77	166	269	502
61	78	167	270	506
62	79	168	271	508
63	80	169	272	512
64	81	170	273	516
65	82	171	274	518
66	83	172	275	522
67	84	173	276	526
68	85	174	277	528
69	86	175	278	532
70	87	176	279	536
71	88	177	280	538
72	89	178	281	542
73	90	179	282	546
74	91	180	283	548
75	92	181	284	552
76	93	182	285	556
77	94	183	286	558
78	95	184	287	562
79	96	185	288	566
80	97	186	289	568
81	98	187	290	572
82	99	188	291	576
83	100	189	292	578
84	101	190	293	582
85	102	191	294	586
86	103	192	295	588
87	104	193	296	592
88	105	194	297	596
89	106	195	298	598
90	107	196	299	602
91	108	197	300	606
92	109	198	301	608
93	110	199	302	612
94	111	200	303	616
95	112	201	304	618
96	113	202	305	622
97	114	203	306	626
98	115	204	307	628
99	116	205	308	632
100	117	206	309	636
101	118	207	310	638
102	119	208	311	642
103	120	209	312	646
104	121	210	313	648
105	122	211	314	652
106	123	212	315	656
107	124	213	316	658
108	125	214	317	662
109	126	215	318	666
110	127	216	319	668
111	128	217	320	672
112	129	218	321	676
113	130	219	322	678
114	131	220	323	682
115	132	221	324	686
116	133	222	325	688
117	134	223	326	692
118	135	224	327	696
119	136	225	328	698
120	137	226	329	702
121	138	227	330	706
122	139	228	331	708
123	140	229	332	712
124	141	230	333	716
125	142	231	334	718
126	143	232	335	722
127	144	233	336	726
128	145	234	337	728
129	146	235	338	732
130	147	236	339	736
131	148	237	340	738
132	149	238	341	742
133	150	239	342	746
134	151	240	343	748
135	152	241	344	752
136	153	242	345	756
137	154	243	346	758
138	155	244	347	762
139	156	245	348	766
140	157	246	349	768
141	158	247	350	772
142	159	248	351	776
143	160	249	352	778
144	161	250	353	782
145	162	251	354	786
146	163	252	355	788
147	164	253	356	792
148	165	254	357	796
149	166	255	358	798
150	167	256	359	802
151	168	257	360	806
152	169	258	361	808
153	170	259	362	812
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156	173	262	365	822
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159	176	265	368	832
160	177	266	369	836
161	178	267	370	838
162	179	268	371	842
163	180	269	372	846
164	181	270	373	848
165	182	271	374	852
166	183	272	375	856
167	184	273	376	858
168	185	274	377	862
169	186	275	378	866
170	187	276	379	868
171	188	277	380	872
172	189	278	381	876
173	190	279	382	878
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175	192	281	384	886
176	193	282	385	888
177	194	283	386	892
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181	198	287	390	906
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185	202	291	394	918
186	203	292	395	922
187	204	293	396	926
188	205	294	397	928
189	206	295	398	932
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193	210	299	402	946
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203	220	309	412	978
204	221	310	413	982
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206	223	312	415	988
207	224	313	416	992
208	225	314	417	996
209	226	315	418	998
210	227	316	419	1000

Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 96/00627

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C07H21/04 C12N9/24 C12N15/56

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 18974 (CHEMGEN CORP) 12 December 1991 see page 1, ln 3; page 7, lns 22-28.	1-15
Y	WO,A,95 18219 (GIST-BROCADES N.V.) 6 July 1995 see the whole document --- -/--	1-15

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 February 1997

Date of mailing of the international search report

11. 03. 97

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NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Osborne, H

INTERNATIONAL SEARCH REPORT

International Application No
PLI/CA 96/00627

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE WPI Week 9444 Derwent Publications Ltd., London, GB; AN 94-353745 XP002026073 "nucleic acid amplification in presence of poly:amine - increases efficiency by removing inhibition caused by impurities in biological sample" & JP,A,06 277 061 (SHIMADZU CORP) see abstract</p> <p style="text-align: center;">---</p>	1-15
Y	<p>WO,A,95 14770 (PACIFIC ENZYMES LTD) 1 June 1995 page 11, paragraph 3 - page 21. see page 4, paragraph 2 - page 6, paragraph 5</p> <p style="text-align: center;">---</p>	1-15
Y	<p>EP,A,0 517 418 (TAKARA SHUZO CO. LTD.) 9 December 1992 see the whole document</p> <p style="text-align: center;">---</p>	1-15
Y	<p>MICROBIOLOGY, vol. 141, July 1995, pages 1731-8, XP000618229 MUNRO G ET AL: "A gene encoding a thermophilic alkaline serine proteinase from Thermus sp. strain Rt41A and its expression in Escherichia coli" see the whole document</p> <p style="text-align: center;">---</p>	1-15
T	<p>WO,A,95 34662 (GIST-BROCADES B.V.) 21 December 1995 see page 5 - page 29</p> <p style="text-align: center;">-----</p>	1-15

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/CA 96/00627

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WO 9118974 A	12-12-91	AU 8060091 A US 5551515 A US 5476775 A	31-12-91 03-09-96 19-12-95
WO 9518219 A	06-07-95	AU 1415095 A BR 9405934 A EP 0686193 A FI 953920 A JP 8507221 T NO 953312 A	17-07-95 26-12-95 13-12-95 21-08-95 06-08-96 19-10-95
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EP 517418 A	09-12-92	JP 5176766 A JP 5284971 A JP 5305000 A US 5436326 A	20-07-93 02-11-93 19-11-93 25-07-95
WO 9534662 A	21-12-95	AU 2882495 A CA 2168344 A EP 0716702 A FI 960631 A NO 960567 A PL 312962 A	05-01-96 21-12-95 19-06-96 12-04-96 14-02-96 27-05-96